
The nucleotide sequence of poliovirus type 3 leon 12 a₁b: comparison with poliovirus type 1

Glyn Stanway, Alan J.Cann, Rudolf Hauptmann*, Pamela Hughes, Lindsay D.Clarke, Roger C.Mountford; Philip D.Minor¹, Geoffrey C.Schild¹ and Jeffrey W.Almond

Department of Microbiology, University of Leicester, Leicester LE1 7RH, and ¹National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, UK

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ABSTRACT

The complete nucleotide sequence of the genome of the Sabin vaccine strain of poliovirus type 3 (P3/Leon 12 a₁b) has been determined from cDNA cloned in *E. coli*. The genome comprises a 5' non-coding region of 742 nucleotides, a large open reading frame of 6618 nucleotides (89% of the sequence) and a 3' non-coding region of 72 nucleotides. There is 77.4% base-sequence homology and 89.6% predicted amino-acid homology between types 1 and 3. Conservation of all glutamine-glycine and tyrosine-glycine cleavage sites suggests a mechanism of polyprotein processing similar to that established for poliovirus type 1.

INTRODUCTION

Polioviruses, the causative agents of paralytic poliomyelitis, are members of the enterovirus group of the family picornaviridae [1]. The three distinct serotypes, 1, 2 and 3, cause identical disease and are closely similar in structure and composition. In each case the 27nm diameter icosahedral capsid consists of 60 copies each of four virus polypeptides, VP1-VP4, surrounding a single-stranded, positive-sense RNA genome of approximately 7,500 nucleotides [2]. The RNA contains a small virus-coded protein, VPg, covalently linked to its 5'-terminus [3,4] and a poly-A tract at its 3'-terminus [5]. The genome is most probably expressed as a single translational unit encoding a large precursor polyprotein which undergoes specific cleavage to give rise to recognized structural and non-structural virus polypeptides [6,7].

Over the past twenty years epidemic poliomyelitis has been almost eliminated from developed countries, mainly through the use of vaccines. The most widely adopted of these are based on

the live-attenuated strains developed by Dr. Albert Sabin in the 1950's [8]. Although these vaccines have been very successful, a low incidence of disease persists in vaccinated communities and there is evidence that instability of the type 3 vaccine may be a contributing factor [9]. Gene cloning and nucleic-acid sequencing studies offer a better understanding of the molecular basis of attenuation of live-vaccines and may suggest ways in which they can be improved.

We have previously described the molecular cloning of poliovirus type 3 using a method based on RNA.cDNA hybrids [10]. This paper describes the complete nucleotide sequence of the type 3 Sabin vaccine, P3/Leon 12 a₁b, derived from these clones. The nucleotide sequence is compared with those previously published for type 1 [7,11,12].

MATERIALS AND METHODS

Virus

A plaque-purified derivative of the P3/Leon 12 a₁b Sabin vaccine strain, isolate #411, was used in these experiments [13,14]. Details of the molecular cloning of this virus have been published previously [10].

Nucleotide sequence analysis

Overlapping cDNAs contained in plasmid vector pAT 153 were sub-cloned into bacteriophage M13 mp7, mp8 and mp9, prior to sequence determination [15]. The cDNAs were purified from vector DNA by digestion with Pst I and electrophoresis on agarose gels. After electrophoretic elution from the gel, the cDNAs were digested with Alu I, Hae III or Sau 3A to provide overlapping fragments for sub-cloning [10].

Nucleotide sequence was obtained using the dideoxynucleotide chain-termination method [16] and the data was collated and assembled with the aid of a Digital PDP 11/44 computer using programs developed by Staden [17].

Other methods

Restriction endonuclease digestions were carried out according to manufacturers instructions. Ligations, restriction mapping and preparation of DNA was as described previously [10].

RESULTS AND DISCUSSION

Overlapping cDNA clones, together spanning the entire genome of poliovirus P3/Leon 12 a₁ b, were characterized by restriction endonuclease mapping, using the method of Smith and Birnstein [18]. The results obtained (not shown) determined our strategy for sub-cloning into bacteriophage M13 prior to nucleotide sequencing. This detailed restriction map, together with the close homology to poliovirus type 1 in nucleotide and/or predicted amino-acid sequence over most of the genome, allowed the relative location of individual gel readings to be discerned. Where corresponding sequence data were obtained from more than one cDNA clone, perfect matching was observed, providing further evidence of the high fidelity of the RNA.cDNA hybrid cloning method [10]. The total sequence of 7432 nucleotides of the P3/Leon 12 a₁ b genome derived from cloned cDNA is presented in figure 1. We are confident that the sequence presented is accurate. All regions were covered by at least two independent sequencing reaction and comparison with published poliovirus type 1 sequence [11] provided a useful check on reading-frame errors. Furthermore, the sequence has been verified by comparison with cDNAs from closely related type 3 strains of almost identical sequence [14].

The calculated molecular weight of the genome is 2.66×10^6 and the proportions of the four nucleotides are A=28.9%, G=23.5%, C=23.4%, U=24.1%. Computer-assisted analysis of the sequence in both polarities reveals just one open reading-frame large enough to encode the known virus proteins [19]. This spans 2206 consecutive codons starting at position 743 and ending with two stop codons in tandem at position 7361.

Homology with poliovirus type 1

The complete nucleotide sequences of two strains of poliovirus type 1 have been determined in three independent laboratories [7,11,12]. These data, together with analyses of infected-cell and virus-structural polypeptides including N and C-terminal sequencing, has allowed a detailed genetic map of poliovirus type 1 to be established [6,7,20,21,22]. This information has aided greatly the interpretation of the poliovirus type 3 sequence presented here, since, based on homology, the two

P3/LEON 12 A B/56 T T A A A C A G C T C T G G G G T T G T T C C C A C C C A G A G G C C C A G T G G C G C T A G T A C T G G T A T C A C G G T A C C T T T G T A C G C C T G T T T T A T A C T C C C C T C C C C
 10 20 30 40 50 60 70 80 90 100

C G C A A C T T A G A A G C A T A C A A T T C A A G C T C A A T P A G A G G G G T G C A A G C A G C G C C T C C G T G G C A A G C A C T A C T G T T T C C C G G T G A G C G C C A T A G A C T T T C C A C G G T T G A A A G G T G T
 110 120 130 140 150 160 170 180 190 200 210 220

C C G A T C C G T T A T C C G C T C A T G T A C T T C A G A A C C T T A G T A T C G C T C T G G A A T C T T C G A G C G T T G C G C T C A G C A C T C A A C C C G G A G T A G C T T G G G C C G A T G A G T C T G G A C A G T C C C C
 230 240 250 260 270 280 290 300 310 320 330 340

A C T G G C G A C A G T G T C A G G C T G C G G C C C A C C T G T G C C C A A A G C C A G C G G A C G C T A G T T G T G A A C A G G G T G T G A A G A G C C T A T T A G C T A C A T A G A G A T C T C C G C G C C C C T G A
 350 360 370 380 390 400 410 420 430 440 450 460

A T G C G G C T A A T T C T A A C C A T G G A G C A G C T G C A A C C A G C C A A G T C C G T G C G G G A A C C G A C T A C T T T G G G T G T C G G T T T C C T T T T A T T C T T G A A T
 470 480 490 500 510 520 530 540 550 560 570 580

G G C T G C T T A T G G T G A A T C A T A G A T T G T T A T C A T A A A G G A G T T G G A T T G G C C A T C C A G T T A A T T A C T C C C T T G T T G T T G A T C C A C T C C C G A A A C G T T T T A C T C T
 590 600 610 620 630 640 650 660 670 680 690 700

T A C T T A T T G A A T T G T T G A A G A C A G G A T T T C A G T G T C A C A A T G G G A C T C A A G T A T C A T C C C A A A A G T A G C G C T C A C G A G A A T T C T A A C C G A G C C T A C G G T G G T T C T A C G A T C A A C
 710 720 730 740 750 760 770 780 790 800 810 820

Y T T I N Y K D S A S N A A S K Q D Y S Q D P S K F T E P L K D V L I K T A P
 830 840 850 860 870 880 890 900 910 920 930 940

A L N I S P N V E A C G Y S D R V L Q L T L G N S T I T T Q E A A N S V V A Y G R
 950 960 970 980 990 1000 1010 1020 1030 1040 1050 1060

W P E F I R D D E A N P V D Q P T E P D V A T C R F Y T L D T V M W G K E S K G
 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180

W W W K L P D A L R D M G L F G Q N M Y Y H Y L G R S G Y T V H V Q C N A S K F
 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300

H Q G A L G V F A I P E Y C L A I G G D S D K Q R Y T S Y A A N A N P G E R G G K F Y
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420

S O F N K D N A V T S P K R E F C P V D Y L L G C G V L L G N A F V Y P H Q I I
 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540



N L R T N N S A T I V L P Y V N A L A I D S M V K H N N W G I A I L P L S P L D
 AATCTGAGCAACAGCAGCAATATGTCTTACCATATGTGAATGCTTTGGCCATGATTCATGGGCAATGGCCATTCGCCCTATCCACCGCTGGAT
 1530 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660

F A Q D S V E I P I T V T I A P M C S E F N G L R N V T A P K F Q G L P V L N
 TTTGCTCAAGATTCACTAGTTGAAATTCACATTTGCCAAATGTAGCCGAGTTCACAGCCCTGCGAACCTGACCTGCAATTAATTCAGGACATACCAGTGTTCGAC
 1670 1680 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780

T P G S N Q Y L T S D N H Q S P C A I P E F D V T P P I D I P G E V K N M M E L
 ACTCTGGTAGAACAGTACCTGCTGACAGACACCAATCACCATGGCAATCCAGAAATTTGATGTACCTCCGCTATGATATCCAGGTGAGGTAAATAACATGATGGAGCTC
 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900

A E I D T M I P L N L E S T K R N T M D M Y R V T L S D S A D L S Q P I L C L S
 GCGGATGACACCATTCTCTCAATTTGGAGAGCACCAGAAACACAAATGGACATGTACAGTTCCTGAGCAGCTCCGATCTATTCGCAACCAATTTGTGCTTCTCA
 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020

L S P A F D P R L S H T M L G E V L N Y Y T H W A G S L K F T F L F C G S M M A
 CTATCCCAGCATTTGATCCGGCTTGTACACACCATCTTGGGAAGTACTGAATATTAATCTAGTGGCGGTCTTGAATTTACCTCTCTGTGTGTTCAATGATGGCT
 2030 2040 2050 2060 2070 2080 2090 2100 2110 2120 2130 2140

T G K I L V A Y A P P G A Q P T S R K E A M L G T H V I W G L G L Q S S C T M
 ACGGGAAATCTAGTGGCCATGACACACAGTGCACACCCCCACAGCCGTAAGAGGCTATGTGGGCACACATCTCATTTGGGATCTTTGGCTGGCAATCATCTGTACTATG
 2150 2160 2170 2180 2190 2200 2210 2220 2230 2240 2250 2260

V V P W I S N V T Y R Q T T Q D S F T E G G Y I S M F Y Q T R I V V P L S T P K
 GTGGTCCGGTGAATTAGTATGTGACATACAGACACTACACAAGATGTTTCACCTGAGGGCGGATATATCAGCATGTTCTACCAACAAGAAATTTGTGTGCCACTGTCCACCCCTAAG
 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380

S M S M L G F V S A C N D F S V R L L R D T T H I S Q S A L P Q I E D L I S E
 AGTATGAGCATGCTGGGTTTGTGTCAGCTGTAATGATTTTCAGTGTGGATTCCTCGGAGACACCACTCACATTTCAAACTCTGCGTTCACAGGTTATGGAAGATTTGATTTCTGAA
 2390 2400 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500

V A Q G A L T L S L P K Q Q D S L P D T K A S G P A H S K E V P A L T A V E T G
 GTTGCACAGGGCCCTAATTTGCTCCGACACAGGATGACTTACCTGATACATAAGCCAGTGCCTCCGCGCCATTCCAAGGAGTACCTGCACCTCAGTCGAGACATGGA
 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620

A T N P L A P S D T V Q T R H V V Q R R S R S E S T I E S F F A R G A C C V A I I
 GCCACCAATCTCTGCGACCATCCGACAGTTCAAACCGCCACGCTAGTCCACAGCAGGAGTCCACATAGAAATCATTTCCGACGGGGGGCTGCGCTATTAAT
 2630 2640 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740

E V D N E Q P T T R A Q K L F A M W R I T Y K D T V Q L R R K L E F T Y S R F
 GAGGTGGAACAATGAACCAACCCGGCACAGAACTATTTGCCATGTGGCCATACATACAAAAGATACAGTCCAGTTCGCGCTTAAGTTGGAGTTTTTCACACTACTCTCGTTT
 2750 2760 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860

D M E F T F V V T A N F T N A N N G H A L N Q V Y Q I M Y I P G A P T P K S W
 GACATGGAAATCCCTTGTGTAACCGCCCACTTCCACACCCCTAATAATGGCCATGCACCTCAACAGGTGTACCAATATGATATCCCCCGAGGGCCACCCACCAAAAGTCAATGG
 2870 2880 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980

D D Y T W Q T S N P S I F Y T Y G A A P A R I S V P Y V G L A N A Y S H F Y D
 GACGACTAGCATTTGGCAACATCTCCAAACCGTCCATATTTTACACCTATGGGCTGCGCCGCGGAACTCAGTGCCTACCTGCTGGGTTAGCCAAATGCTTACTCGCACTTTTACGAC
 2990 3000 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100

G F A K V P L K T D A N D O I G D S L Y S A M T V D D F G V L A V R V V N D H N
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 3110 3120 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220

P T K V T S K K V R I Y M K P K H V R V W C P P R P P R A V P Y Y G P G V D Y R N N
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 3230 3240 3250 3260 3270 3280 3290 3300 3310 3320 3330 3340

L D P L S E K G L T T Y G F G H O N K A V Y T A G Y K I C N Y H L A T K E D L O
 TTGACCCCTTATCTGAGAAAGGTTGACCACATATGGCTTGGGCATCAGAAATAAGCTGTGTACAGTCTGGTGTACAAAGTCTGCAATCCATCTCGCCACTAAGAGGATTTACAA 3460
 3350 3360 3370 3380 3390 3400 3410 3420 3430 3440 3450 3460

N A V S I M W N R D L L V V E S K A Q G T D S I A R C N C N A G V Y Y C E S R R
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 3470 3480 3490 3500 3510 3520 3530 3540 3550 3560 3570 3580

K Y P V S F V G P T F Q Y M E A N D Y Y P A R Y Q S H M L I G H G F A S P G D
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 3590 3600 3610 3620 3630 3640 3650 3660 3670 3680 3690 3700

C G G I L R C Q H G V I G I V T A G E G L V A F S D I R D L Y A Y E E A M E
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 3710 3720 3730 3740 3750 3760 3770 3780 3790 3800 3810 3820

O G I S N
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 3830 3840 3850 3860 3870 3880 3890 3900 3910 3920 3930 3940

L L K N L I K I I S S L V I I T R N Y E D T T T V L A T L A L L G C D V S P W Q
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 3960 3970 3980 3990 4000 4010 4020 4030 4040 4050 4060 4070

W L K K K A C D T L E I P Y V I R O G D S W L K K P T E A C N A A K G L E W V S
 TGGCTGAAGAANGAATGTGACATTTGGAGATTCCCTATGTTATAGACAGGGGTGATGGTTGGAATAATTTACTAGGGCTGCACCCGACTAAGGGGTGGAATGGGTGTGCC 4180
 4070 4080 4090 4100 4110 4120 4130 4140 4150 4160 4170 4180

N K I S K F I D W L R E R I I P O A R D K L E F V T K K L E M L E N Q I S T
 AACAAATCTCAAAATTTATGACTGTTGAGAGAAAGAAATCAATCCCAAGCCAGGCAAGCTTGAGTTTGTAAACCAATTTGAAACAGTTGGAAATGCTAGAGAACTCAGATATCCACA 4300
 4190 4200 4210 4220 4230 4240 4250 4260 4270 4280 4290 4300

I H O S C P S Q E H O E I L F N N V R L S I O S K R F A P L Y A L E A K R I Q
 ATACCAATCTTGTCCAAGTAGGAACACAGGAAATTTTGTCAACAATGTACGCTGGTGTCCATCAATCCAAAGAGATTCGCTCCATTTGACCGACTTGGCCCAAGAGATACAA 4420
 4310 4320 4330 4340 4350 4360 4370 4380 4390 4400 4410 4420

K L E H T I N N Y I O F K S K H R I E P V C L L V H G S P G T G K S V A T N L I
 AAGTTGGAACACACCATTATTAATACATACAGTTCAAGCAACACCCGATTTGAGCCAGTATGGGCCCCAGTACAGGAAATCAGTTGCCACTACCTAATTT 4540
 4430 4440 4450 4460 4470 4480 4490 4500 4510 4520 4530 4540

A R A I A E K E N T S T Y S L P P D P S H F D G Y K Q Q G V I M D D L N Q N P
 GCTAGAGCCATAGCTGAGAAAGAGAACACCTCCACCCTACCTCGCTACCACCGGACCGGTCTCACTTTGATGGATACAAACAAGGTTGGTTATCATGGACGACCTAAACCAAAACCCCG 4660
 4550 4560 4570 4580 4590 4600 4610 4620 4630 4640 4650 4660

D G A D M K L F C Q M V S T V E F I P P M A S L E E K G I L F T S N Y V L A S T
 GATGGGCAGATA TGAAGCTCTTTTGTCAAATGGTGTCCAC TGTGGAGTTATCCCACTATGGCC TGGTGAAGAGAAAGCCATTCGTTCACATCAAC TATGTTTATAGCCTCCACC
 467# 468# 469# 470# 471# 472# 473# 474# 475# 476# 477# 478# 479# 480# 481# 482# 483# 484# 485# 486# 487# 488# 489# 490#

N S S R I T P T V A H S D A L A R R F A F D M D I Q V M G E Y S R D G K L N M
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 479# 480# 481# 482# 483# 484# 485# 486# 487# 488# 489# 490#

A M A T E T C K D C H Q P A N F K R C C P L V C G K A I O L M D K S R V R Y S
 GCAATGGCTACGAGACGTCAGGACTGCCACCAACAGCAACATTCAAAGATGCTGCTTATGCTGTGTGTAAGCAATTCAGTAATGGACAAATCTCCAGAGTTAGGTACAGT
 491# 492# 493# 494# 495# 496# 497# 498# 499# 500#

V D Q I T T M I I C A N E R N R R S N I G N C M E A L F Q G P L O Y K D L K I D I K
 GTTGACAGATCTACTACAAATGATTAACACGAGAGAAACAGAAATCAACATGGCAATTCGATGGAGCTTTGTTCCAAAGCACCTCCAGTCAAGACCTGAAATTTGACATCAAG
 503# 504# 505# 506# 507# 508# 509# 510# 511# 512# 513# 514#

T R P P P E C I N D L L Q A V D S Q E V R D Y C E K K G W I V N I T S O V Q T E
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 515# 516# 517# 518# 519# 520# 521# 522# 523#

R N I N R A M T I L O A V T T F A A V A G V Y V M Y K L F A G H Q G A Y T G L
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 527# 528# 529# 530# 531# 532# 533# 534# 535#

P N K R P N V P T I R A A K V Q G P G F D Y A V A M A K R N I V T A T T S K G E
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 539# 540# 541# 542# 543# 544# 545# 546# 547# 548# 549# 550#

F T M L G V H D N V A I L P T H A S P G E S I V I D G K E V E I L D A K A L E D
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 551# 552# 553# 554# 555# 556# 557# 558# 559# 560# 561# 562#

Q A G T N L E I T I T L K R N E K F R D I R Q H I P T Q I T E T N D G V L I V
 CAGGAGCCTAACTCGMAATCACCATAAACCTCAAAGAATAAGAAATTCAGAGATATCAGACAAACATACCCACCTCAAACTCCGAGACCAATGATGGAGTCTGATTTGTG
 563# 564# 565# 566# 567# 568# 569# 570# 571# 572# 573# 574#

N T S K Y P N M Y P V G A V T E Q G Y L N L G R Q T A R I L M Y N F P T R A
 AACACTAGTAAAGTCCCAACATGATGTTCTGCTGCTGACTAGCAGGATACCTAAATCTCGGTGGCCGACACTCTCGTATTTCTAATTTACAACTTTCCAAACAGAGCT
 575# 576# 577# 578# 579# 580# 581# 582# 583# 584# 585# 586#

G O C G G V I T C T G K V I G M H V G G N G S H G F A A L L K R S Y F T O S Q G
 GGTCAAGTGTGGAGTACATGCATGCATGCGGAATGCAAGTGTGGGAATGGTTCACATGGGTTTGCACGCCCTGAAGCCGCTCACTACTACAGACCCAAGT
 587# 588# 589# 590# 591# 592# 593# 594# 595# 596# 597# 598# 599#

← POLYM
 GAAATCCAGTGGATGAGACCATCAAGGAGCCGGTATCCAAATTAAGCCGCCCAACCAAGACCAAGCTCGAGCCCGCCCTTCCACTATGTTTGAAGGATTAAGGAACCAAGCA
 599# 600# 601# 602# 603# 604# 605# 606# 607# 608# 609# 610#

V L T K N D P R L K T D F E E A I F S K Y V G N K I T E V D E Y M K E A V D H Y
 GTCCTCAAAAAGATGATCCAGACTAAAACAGACTTTGAAGAACAATCTCTCTAAGATATGTAGGGAAACAAGATCACCTGAGTGGATGATACATGAAGAAGCCAGTGGACCTAT
 611# 612# 613# 614# 615# 616# 617# 618# 619# 620# 621# 622#

A G O L M S L D I S T E O M C L E D A M Y G T D G L E A L D L S T S A G Y P Y V
 C T G G C A A C T T A T G T C C T G G A T A T C A G C A C A G A G C A A T G T C T A G A G A C C C C A T G T A T G T A C T A G T G G T C T G A G G C G T A G A T C T C T A C C A G T C C G G G T A C C C T A G C T G
 6230 6240 6250 6260 6270 6280 6290 6300 6310 6320 6330 6340

A M G K K K R D I L N K Q T R D T K E M Q R L L D A Y G I N L P L V T Y V K D E
 G C A A T G G G A A G A A G A G A G A T A C C T A A C A A G C A A C C A G A C A C C A A A G A A T G C A A G A C T T T T G G A C G C T P A C G G A A T C A A C C T A C C A T T A G T G A C A T A T G C A A G G A G A G
 6350 6360 6370 6380 6390 6400 6410 6420 6430 6440 6450 6460

L R S K T K V E Q G K S R L I E A S S L N D S V A M R M A F G N L Y A A F H R N
 C T G A G T C C A A A C A A A G T G G A A C A G G A A A T C C A G A C T G A T T G A A G C T T C C A G T C T A A T A G T C A G T G G C C A G A A T G G C A T T T G A A A C C T T T A T C A G C A T T C C A C A G A A T
 6470 6480 6490 6500 6510 6520 6530 6540 6550 6560 6570 6580

P G V V T G S A V G C D P D L F W S K I P V L M E E K L F A F D Y T G Y D A S L
 C C A G G G T C T C A C T G T A G T G C A T C C A G A C C T A T T C T G G A G C A A G A T C C C A G T G T A T G A T G G A A A A G C T A T T T G C C T T T G A T T A C A C A G G A T A C G A C G C A T C A C T T
 6590 6600 6610 6620 6630 6640 6650 6660 6670 6680 6690 6700

S P A W F E A L K M V L E K I G F G D R V D Y I D Y L N H S H L Y K N K I Y C
 A G C C A G C T T G G T T G A G C A C T A A G A T G T T A G A A A A T T G G T T T G G A G A T A G A G T G G A T T A C A T A G A C T A C C T T A A C C A T T C A C A C C A C T T G T A C A A A A C A A G A T A T A T T G T
 6710 6720 6730 6740 6750 6760 6770 6780 6790 6800 6810 6820

V K G G M P S G C S G T S I F N S M I N N L I I R T L L L K T Y K G I D L D H L
 G T T A A G G G C G C A T G C C T G C T C G G C A C T T C A A T T T T T A A T T C A A T T A A C A A T T T G A T C A T T A G C C G C T T T T A C T G A A A A C C T A C A A G G C C A T A G A T T T G G A C C A C T T A
 6830 6840 6850 6860 6870 6880 6890 6900 6910 6920 6930 6940

K M I A Y G D D V I A S Y P H E V D A S L L A Q S K D Y G L T M T P A D K S A
 A A A A T G A T T G C C T A T G T G A C G A T G A A T A G C T T C C C A T G C C C A T G A G G T T G A C G T A G T C T C C P A G C C C A A T C A G A A A A G A C A T A T G G A C T A A C C A T G A C T C C G G C A G A T A A A T C T G C C
 6950 6960 6970 6980 6990 7000 7010 7020 7030 7040 7050 7060

T F E T V T W E N V T F L K R F P R A D E K Y P F L I H P V M P M K E I H E S I
 A C T T T T G A G A C A C A T G G G A A T G T A A C T T T C T T G A A A A G A T T C T C A G A C A G A T G A A A T A C C C C T C C T C A T A C A T C C A G P A A T G C C A A T A A G G A A A T T C A T G A A T C A A T C
 7070 7080 7090 7100 7110 7120 7130 7140 7150 7160 7170 7180

R W T K D P R N T Q D H V R S L L C L L A W H N G E E A Y N K F L A K I R S V P I
 A G A T G C A A A A G A T C T C G G A A T A G C A C C A T G C T C C T T G T C T A T T G C C T T G G C A C A C G G G A A G A A T A C A C A A A T T T T T A G C T A A A A T T A G G A T T A G G A T G G C C C A A T C
 7190 7200 7210 7220 7230 7240 7250 7260 7270 7280 7290 7300

G R A L L L P E Y S T L Y R R W L D S F * *
 G G A A G C T T T T G T C C A G A T A C T A A C A T T G T A C C G C G C T T G C C T T G A C T A T T T T A G T A A C C C T A C C T C A G T C G A A T T G G A T T G G G T C A T A C T G T T A G G G T A A A T T T T T C T
 7310 7320 7330 7340 7350 7360 7370 7380 7390 7400 7410 7420

T T A A T T C G G A G G - P O L Y A
 7430

sequences can be aligned almost perfectly. The overall homology in nucleotide sequence between the two types is 77.4%, higher than some estimates [23] but in agreement with the recent results obtained using a method based on cDNA hybridization [24]. The size of the 5' and 3' non-coding regions are identical to type 1 at 742 and 72 bases respectively, but the coding region of type 3 contains three fewer triplets than that of type 1.

Non-coding regions

The sequence of the 5'-terminus of the cDNA presented in fig. 1 is identical to the previously published sequence of 20 nucleotides determined directly from genomic RNA of poliovirus type 3 [25]. This sequence, plus 15 succeeding nucleotides up to a Hae III recognition site, has been determined from five independent cDNA clones and is in each case immediately preceded by a homopolymer tract of about 20 dG residues. This confirms that the 5'-sequence of the cDNA depicted in fig. 1 actually corresponds to the 5'-terminal sequence of poliovirus type 3 genomic RNA, and that the RNA.cDNA hybrid method permits direct cloning of the 5'-terminus [10].

The 5' non-coding region shows close homology to poliovirus type 1. There is only one difference in the first 54 nucleotides (A at position 22 in type 1). This difference forms part of the loop in an otherwise totally conserved stable hairpin structure previously recognized for type 1 and postulated to play a role in ribosomal recognition or in polymerase binding [26]. From position 54 until the start of the large open reading-frame at position 743, the overall homology is approximately 80%. There is a notable stretch of 65 identical bases from 509-573, the significance of which remains obscure. Although it appears that several insertions and deletions have occurred in this 5' region, the size, 742 nucleotides, is identical in the two types. Before the start of the 2206 codon reading-frame at

Figure 1. Complete Nucleotide sequence of the genome of poliovirus type 3 Leon 12 a₁b as determined from cloned cDNA, plus the predicted amino-acid sequence from the major open reading frame. Cleavage sites analagous in location to those used in poliovirus type 1 are indicated.

Table 1. Codon usage in the long open-reading frame of poliovirus type 3. STOP = termination codons.

<u>2nd</u>	<u>U</u>		<u>C</u>		<u>A</u>		<u>G</u>		<u>3rd</u>	
<u>1st</u>	<u>U</u>	F	40	S	18	Y	36	C	23	<u>U</u>
			43		38		66		18	<u>C</u>
		L	21		44	STOP	0	STOP	0	<u>A</u>
			45		7		0	W	27	<u>G</u>
	<u>C</u>	L	17	P	22	H	17	R	8	<u>U</u>
			27		21		31		13	<u>C</u>
			32		59	Q	54		6	<u>A</u>
			34		16		37		3	<u>G</u>
	<u>A</u>	I	65	T	55	N	49	S	25	<u>U</u>
			43		43		61		22	<u>C</u>
			23		47	K	69	R	49	<u>A</u>
		M	65		10		55		18	<u>G</u>
	<u>G</u>	V	28	A	49	D	59	G	43	<u>U</u>
			24		44		60		25	<u>C</u>
			26		59	E	46		36	<u>A</u>
			67		16		64		38	<u>G</u>

position 743, there are seven other AUG codons which could be used to initiate translation. Most of these establish reading-frames which are not conserved free of nonsense-codons between poliovirus types 1 and 3 and are therefore of doubtful significance. There is one conserved potential reading-frame located at 239-322 which could code for a peptide of 28 amino-acids. However, there is at present no evidence that this region is translated in poliovirus-infected cells.

The non-coding region at the 3'-terminus of the genome is identical in sequence to that determined for poliovirus type 1 Mahoney as has been discussed previously [10]. Although the conservation of this region implies that its primary structure is functionally important, it is noteworthy that other enteroviruses show substantial divergence in this region, [27, our unpublished work].

Coding region

The open reading-frame from nucleotide 743 to 7360 comprises 2206 consecutive codons and is the only region of the genome which can encode the known virus polypeptides [19]. This single reading-frame probably represents the entire coding function of

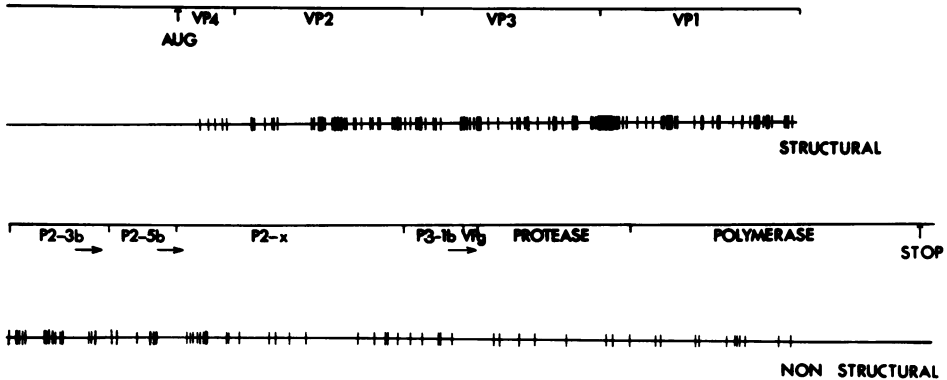


Figure 2. Diagrammatic representation of the amino-acid sequence differences between poliovirus type 3 Leon 12 a b and poliovirus type 1 (Mahoney strain). A single vertical line represents a single amino-acid substitution, insertion or deletion. Lines of double thickness represent two adjacent changes, etc. The map of the genome is based on that determined for poliovirus type 1 [7].

the genome. The AUG at position 743 is the eighth such codon from the 5'-terminus, so presumably a mechanism exists which allows ribosomes to bypass the preceding seven. It is noteworthy that poliovirus mRNA lacks a 5'-terminal cap [28,29] and therefore its translation is likely to be initiated by a mechanism different from that used by cellular mRNA [30].

Codon usage is illustrated in table 1 and shows only minor differences from that of poliovirus type 1 [11]. The overall homology in predicted amino-acid sequence is 89.6% although there is substantial variation around this figure between individual virus proteins. Figure 2 illustrates the distribution of amino-acid sequence differences between types 1 and 3. Most are the result of point mutations but there are also several insertions and/or deletions of whole triplets. In general non-structural proteins are more highly conserved than structural proteins. This may be due to a greater selection pressure for structural variants of the virus particle e.g. immunological, or may simply reflect a lower sequence flexibility in non-structural proteins whose role is mainly enzymatic.

The largest region showing no homology to type 1 spans

nucleotides 2480-2542 encoding the N-terminus of VP1 and possibly the C-terminus of VP3. This sequence contains two glutamine-glycine potential proteolytic-cleavage sites at positions 2477 and 2509, either or both of which may be used to generate VP1 and the VP0-VP3 precursor [7]. Amino-acid sequence analysis of the C-terminus of VP3 and the N-terminus of VP1 is in progress to resolve this question.

Another region of substantial sequence divergence includes the major antigenic site against which neutralizing antibodies are directed (encoded by nucleotides 2754-2776) [31]. We have previously shown that this sequence of eight amino-acids in the VP1 protein plays a critical role in the neutralization of poliovirus type 3 [32]. Because of the similarity in sequence over most of VP1 between the serotypes, it is likely that the eight analogous amino-acids from poliovirus type 1 constitute its equivalent antigenic site. Five differences out of the eight amino-acids comprising this region is a level of homology consistent with the serological distinctness of the two types. The relative similarity in sequence arrangement however, (also for type 2, A. Nomoto personal communication), suggests that the structure of this site may be less flexible than, for example, the equivalent region in the VP1 protein of foot-and-mouth disease virus, where more drastic variations probably account for the greater number of serotypes of this virus [33].

As discussed previously [10], the putative protease (P3-7c) [34] encoded by nucleotides 5428-5944 is highly conserved between types 1 and 3 (98% amino-acid homology), suggesting an identical function. Conservation of all glutamine-glycine pairs including those shown to be involved in processing of the type 1 polyprotein [7] (fig. 3) lends further support to this conclusion. Similarly all tyrosine-glycine pairs and the asparagine-serine cleavage site between VP4-VP2 are conserved, suggesting that host involvement in processing is identical in the two types [34].

Concluding remarks

The complete nucleotide sequence presented here is the first to be derived from a poliovirus type 3 strain. Knowledge of this sequence will be helpful in many respects. Sequence

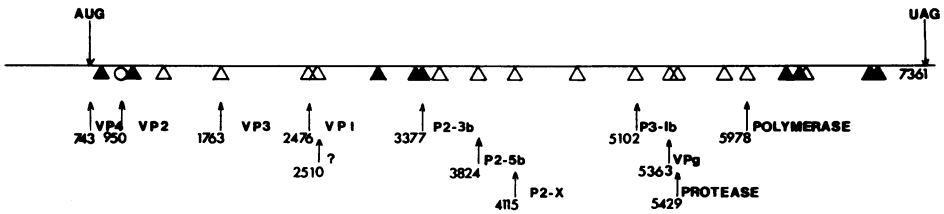


Figure 3. Location of potential proteolytic-cleavage sites in the polyprotein encoded by poliovirus type 3 Leon 12 a₁b. Arrows indicate those sites used in poliovirus type 1 [7]. Proteins indicated are those whose N-termini are generated by the cleavages shown.

- ▲ = tyrosine-glycine
- △ = glutamine-glycine
- = asparagine-serine (only that known to be used, VP2-VP4, is indicated).
- ? = potential alternative site for the generation of VP1, (see text).

information is an essential prerequisite for elucidation of the molecular basis of the biological features of the virus including neurovirulence, antigenicity and tissue-tropism. The possibility of recovering live poliovirus from cloned cDNA has opened up new possibilities for genetic studies on these viruses [35]. The current live-attenuated type 3 poliovirus vaccine has certain disadvantages, particularly with respect to reversion of the virus to virulence on passage in tissue-culture [36] and in the occurrence of vaccine-associated disease [9,37,38]. Manipulation of the genome using techniques such as site-directed mutagenesis, recombination *in vitro* and the insertion or deletion of genetic material, may lead to the production of safer vaccines. Work in this area is currently in progress.

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*Present address: Ernst-Boehringer-Institut für Arzneimittelforschung, Bender and Co. Ges mbH
Wien, Dr. Boehringer-Gasse 5-11, A-1121 Wien, Austria

REFERENCES

1. Matthews, R.E.F. (1979) In Classification and nomenclature of viruses, Ed Karger, S.
2. Putnak, J.R. and Phillips, B.A. (1981) Microbiological Reviews 45, 287-315.
3. Lee, Y., Nomoto, A., Detjen, M. and Wimmer, E. (1977) P.N.A.S. U.S.A. 74, 59-63.
4. Pettersson, R.F., Flanagan, J.B., Rose, J.K. and Baltimore, D. (1977) Nature 268, 270-272.
5. Yogo, Y. and Wimmer, E. (1972) P.N.A.S. U.S.A. 68, 1877-1882.
6. Jacobson, M.F. and Baltimore, D. (1968) P.N.A.S. U.S.A. 61, 77-84.
7. Kitamura, N., Semler, B.L., Rothberg, P.G., Larsen, G.R., Adler, C.J., Dorner, A.J., Emnini, E.A., Hanecak, R., Lee, J.L., van der Werf, S., Anderson, C.W. and Wimmer, E. (1981) Nature 291, 547-553.
8. Sabin, A.B. and Boulger, L. (1973) J. Biol. Stand. 1, 115-118.
9. World Health Organization (1969) Bull. WHO 40, 925-945.
10. Cann, A.J., Stanway, G., Hauptmann, R., Minor, P.D., Schild, G.C., Clarke, L.D., Mountford, R.C. and Almond, J.W. (1983) Nucleic Acids Research 11, 1267-1281.
11. Racaniello, V.R. and Baltimore, D., (1981) P.N.A.S. U.S.A. 78, 4887-4899.
12. Nomoto, A., Omata, T., Toyoda, H., Kuge, S., Horie, H., Katoda, Y., Genba, Y., Nakano, Y. and Imura, N. (1982) P.N.A.S. U.S.A. 79, 5793-5797.
13. Minor, P.D. (1980) Journal of Virology 34, 73-84.
14. Stanway, G., Cann, A.J., Hauptmann, R., Mountford, R.C., Clarke, L.D., Reeve, P., Minor, P.D., Schild, G.C. and Almond, J.W. European Journal Biochemistry, Submitted for publication.
15. Messing, J., Crea, R. and Seeburg, P. (1981) Nucleic Acids Research 9, 319-321.
16. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) P.N.A.S. U.S.A. 74, 5463-5467.
17. Staden, R. (1980) Nucleic Acids Research 8, 3673-3694.
18. Smith, H.O. and Birnstiel, M.L. (1976) Nucleic Acids Research 3, 2387-2398.
19. Nottay, B.K., Kew, O.M., Hatch, M.H., Heyward, J.T. and Obejeski, J.F. (1981) Virology 107, 404-423.
20. Larsen, G.R., Anderson, C.W., Dorner, A.J., Semler, B.L. and Wimmer, E. (1982) J. Virol. 41, 340-344.
21. Semler, B.L., Anderson, C.W., Kitamura, N., Rothberg, P.G., Wishart, W.L. and Wimmer, E. (1982) P.N.A.S. U.S.A. 78, 3464-3468.
22. Emnini, E.A., Elzinga, M. and Wimmer, E. (1982) J. Virol. 42, 194-199.
23. Young, N.A. (1973) Virology 56, 400-403.
24. Tracey, S. and Smith, R.A. (1981) Journal of General Virology 55, 193-199.
25. Nomoto, A., Toyoda, H. and Imura, N. (1981) Virology 113, 54-63.

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26. Larsen, G.R., Semler, B. and Wimmer, E. (1981) *J. Virol.* 37, 328-335.
 27. Porter, A.G., Fellner, P., Black, D.N., Rowlands, D.J., Harris, T.J.R. and Brown, F. (1978) *Nature* 276, 298-301.
 28. Hewlett, M.J., Rose, J.K. and Baltimore, D. (1976) *P.N.A.S. U.S.A.* 73, 327-330.
 29. Nomoto, A., Lee, Y.F. and Wimmer, E. (1976) *P.N.A.S. U.S.A.* 73, 375-380.
 30. Kozak, M. (1978) *Cell* 15, 1109-1123.
 31. Minor, P.D., Schild, G.C., Bootman, J., Evans, D.M.A., Ferguson, M., Reeve, P., Spitz, M., Stanway, G., Cann, A.J., Hauptmann, R., Clarke, L.D., Mountford, R.C., and Almond, J.W. *Nature*, 301, 674-679.
 32. Evans, D.M.A., Minor, P.D., Schild, G.C. and Almond, J.W. (1983) *Nature*, in press.
 33. Makoff, A.J., Paynter, C.A., Rowlands, D.J. and Boothroyd, J.C. (1982) *Nucleic Acids Research* 10, 8285-8295.
 34. Hanecak, R., Semler, B.L., Anderson, C.W. and Wimmer, E. (1982) *P.N.A.S. U.S.A.* 79,
 35. Racaniello, V.R. and Baltimore, D. (1981) *Science* 214, 916-919.
 36. Boulger, L.R., Marsden, S.A., Magrath, D.I., Taffs, L.F. and Schild, G.C. (1979) *J. Biol. Stand.* 7, 97-111.
 37. Cossart, Y.E. (1977) *British Medical Journal* 1, 1621-1623.
 38. Minor, P.D. (1982) *Journal of General Virology* 59, 307-317.